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Cyclosporine A enhances platelet aggregation

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Cyclosporine A enhances platelet aggregation. In view of the reported increase in thromboembolic episodes following cyclosporine A (CyA) therapy, the effect of this drug on platelet aggregation and thromboxane A_2 release was investigated. The addition of CyA, at therapeutic concentrations to platelet rich plasma from normal subjects in vitro was found to increase aggregation in response to adrenaline, collagen and ADP. Ingestion of CyA by healthy volunteers was also associated with enhanced platelet aggregation. The CyA-mediated enhancement of aggregation was further enhanced by the addition in vitro of therapeutic concentrations of heparin. Platelets from renal allograft recipients treated with CyA also showed hyperaggregability and increased thromboxane A_2 release, which were most marked at "peak" plasma CyA concentration and less so at "trough" concentrations. Platelet hyperaggregability in renal allograft patients on long-term CyA therapy tended to revert towards normal following the replacement of CyA with azathioprine. Hypertensive patients with renal allografts on nifedipine therapy had normal platelet function and thromboxane release in spite of CyA therapy. These observations suggest that CyA-mediated platelet activation may contribute to the pathogenesis of the thromboembolic phenomena associated with the use of this drug. The increased release of thromboxane A_2 (a vasoconstrictor) may also play a role in mediating CyA-related nephrotoxicity.

Cyclosporine A (CyA) has an important role in the prevention of rejection in renal transplant recipients [1, 2]. Recently, however, it has been suggested that CyA treatment in this patient group is associated with an increased incidence of thromboembolic complications [3, 4]. Glomerular capillary thrombi have been reported to occur with increased frequency in renal allograft recipients with CyA nephrotoxicity when compared with those who have acute allograft rejection [5, 6].

A recent study from Vanrenterghem et al [3] showed that CyA-treated patients had a number of hemostatic changes which favor thrombosis, and which may therefore account for the observed high incidence of thromboembolic events in these patients. This study, however, left some questions unanswered. For example, although there was a suggestion that CyA levels correlated with ADP-induced platelet aggregation, this was not quantified. Furthermore, despite platelet hyperaggregability no associated change in thromboxane A_2 (TXA_2) was demonstrated.

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The present study attempts to clarify some of these unresolved issues. Thus we have addressed the problem of CyA levels and measured platelet TXA_2 release in response to several agonists in renal transplant recipients. We have also assessed the effect of CyA on platelets under strictly controlled conditions, in vitro and after ingestion by healthy volunteers.

Methods

Study 1: Platelet aggregation following the in vitro addition of CyA to platelets obtained from healthy volunteers

Blood was collected (from an antecubital vein) with minimum stasis from seven healthy volunteers. The blood was anticoagulated with trisodium citrate (9 parts blood: 1 part citrate) as previously described [7]. All the volunteers denied taking any drugs for at least two weeks prior to sampling. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared by centrifugation at room temperature [7]. The maximum delay between sampling and starting centrifugation was 10 minutes. Following centrifugation, PRP was kept at 37°C for all subsequent experiments as cooling is known to alter platelet function [8]. Aggregation was expressed as the percentage fall in optical density three minutes after adding the aggregating agent [7]. The difference in optical density between PRP and PPP was defined as 100%. Aggregations were carried out in Chronolog dual channel aggregometers (Coulter Electronics, Luton, Bedsford, UK) [7].

For each subject, experiments were run in pairs; one aggregometer channel contained PRP with the appropriate volume of saline only (control) and the other channel a solution of CyA (test). CyA was dissolved in a minimum amount of absolute ethanol and then diluted in physiological saline. Appropriate volumes of absolute ethanol were added to the saline control. The final concentration of absolute ethanol in the cuvette was 160 mg/liter. The concentrations of CyA used were selected to reflect the plasma levels in renal transplant patients treated with this drug [9]. The concentrations of the agonists (Table 1) were submaximal (producing 0 to 50% aggregation in the saline controls: ADP, 1 to 2 μmol/liter; adrenaline, 1 to 3 μmol/liter; collagen, 0.15 to 0.50 mg/liter) so as to highlight enhancement [10]. The PRP was first incubated with CyA or saline for two minutes, and aggregation was then induced with the appropriate agonist. In other experiments, CyA (125 to 500 ng/ml) was added to PRP and the baseline tracing followed for up to 15

Table 1. Median (range) percentage platelet aggregation following the in vitro addition of CyA to PRP prepared from healthy controls ($N = 7$)

CyA final concentration ng/ml	Adrenaline-induced aggregation		Collagen-induced aggregation		ADP-induced aggregation	
	Control	CyA	Control	CyA	Control	CyA
125	33←$P < 0.05$→57 (25–44)	(36–69)	0←NS→36 (0–50)	(19–68)	18←NS→44 (11–46)	(18–56)
250	23←$P < 0.01$→63 (10–50)	(40–81)	0←$P < 0.01$→47 (0–47)	(22–73)	13←$P < 0.01$→40 (9–47)	(26–66)
500	26←$P < 0.01$→61 (15–50)	(25–85)	0←$P < 0.01$→59 (0–49)	(28–71)	22←$P < 0.01$→53 (12–49)	(30–72)

P values obtained by comparing control aggregation with aggregation in the presence of CyA (Wilcoxon test)

minutes to establish whether CyA alone could induce aggregation (more than 20% fall in optical density).

Additive-free CyA was from Sandoz Ltd. (Basle, Switzerland).

Study 2: Platelet function following the ingestion of CyA by healthy volunteers

Ten fasting healthy volunteers (7 males; 3 females), median age 28 years (range: 24 to 37 years), from whom informed consent had been obtained, were included in the study. No other drugs had been taken for at least two weeks before the study. CyA (Sandimmun; Sandoz) was administered in a dose of 15 mg per kg body weight. CyA was dissolved in 50 ml of chocolate milk (Unigate Dairies).

Blood was collected and PRP and PPP was prepared as described in Study 1.

Submaximal agonist doses were used and percentage aggregation was calculated three minutes after adding the agonist, as described in Study 1, except that saline or CyA in solution were not added in vitro. Platelet counts in PRP were monitored using a Coulter ZM counter (Coulter).

Plasma CyA concentrations were estimated by an HPLC method as previously described [9, 11].

Four control subjects were sampled after taking the same amount of chocolate milk without CyA.

Study 3: Effect of normal plasma ionic calcium concentrations and therapeutic levels of heparin on CyA-mediated enhancement of platelet aggregation, in vitro

In the experiments described in Studies 1 and 2, blood was collected in citrate. This anticoagulant functions by calcium chelation; ionic calcium levels are therefore very low. It was thus necessary to establish whether CyA-mediated enhancement of platelet aggregation could be observed at normal plasma ionic calcium concentrations. This objective was achieved by preparing PRP using heparin (Thromboliquine, Organon BV, Oss, Holland) as anticoagulant. The procedures and number of control subjects was as described in Study 1, except that the anticoagulant volume was only 4 μl of heparin dissolved in physiological saline to achieve a final concentration of 2 U/ml of blood. Baseline aggregation tracings were found to be unstable and a considerable drift was often noted, even in the absence of aggregating agents. Aggregation patterns were not consistent

Table 2. Median and (range percentage platelet aggregation in heparinized and citrated PRP (N = number of subjects evaluated)

Aggregating Agent	Saline	Heparin 0.5–1.0 U/ml	CyA (500 ng/ml)	CyA + heparin
Heparinized PRP				
ADP ($N = 7$) (0.5 to 1.0 μmol/liter)	34 (13–50)		50[a] (19–75)	
Adrenaline ($N = 3$) (0.5 to 1.0 μmol/liter)	24 (16–36)		45 (38–52)	
Citrated PRP				
ADP ($N = 7$) (1.0 to 1.5 μmol/liter)	9 (2–20)	36[a] (28–60)	18[a] (8–33)	50[b] (32–75)
Adrenaline ($N = 3$) (0.5 to 1.0 μmol/liter)	5 (2–15)	44[a] (18–67)	19[a] (6–48)	71[b] (40–83)

Statistical analysis (Wilcoxon test):
saline vs. CyA or heparin alone: [a] $P < 0.01$
CyA + heparin vs. CyA or heparin alone: [b] $P < 0.01$

except with ADP as the aggregating agent; therefore the seven samples were tested only with this agonist. Three of these samples (those with the most stable baseline tracings) were also evaluated with adrenaline as the aggregating agent. The concentrations of ADP, adrenaline and CyA used are shown in Table 2. The concentrations of aggregating agents (ADP, adrenaline) were set at lower levels than in Study 1 to allow enhancement with CyA and heparin alone and a further enhancement of aggregation when both these drugs are added to PRP.

Blood was also collected in citrate (Study 1) from the same subjects and on the same occasion as the heparinized samples. PRP from these citrated samples was prepared simultaneously with that of the heparinized samples in the same centrifuge. Aggregations were then carried out: heparinized samples first and citrated samples second on one occasion and the sequence reversed on the next occasion. Platelet counts were measured as described in Study 2. The count in the citrated samples was corrected for dilution so that it could be compared with counts in heparinized PRP.

The purpose of simultaneously collecting citrated and heparinized samples was: (a) to establish whether heparin caused a significant fall in platelet count, as has been previously suggested [reviewed in 12]; (b) to compare aggregation responses in citrated and heparinized PRP; (c) to establish whether the enhancing effect of CyA was "additive" to the enhancement which is already known to occur with heparin [10, 12, 13]. This was achieved by adding heparin (final concentration: 0.5 to 1.0 μ/ml, that is, therapeutic concentrations [10]) to citrated PRP; and (d) to compare ionic calcium concentrations in citrated and heparinized PRP. This was achieved using a Radiometer ICA 1 ionic calcium analyzer (Copenhagen, Denmark). Appropriate control and calibration samples (supplied by the manufacturers) were used before and after each measurement.

Study 4: Platelet function in renal allograft recipients taking CyA

Renal allograft recipients (8 males; 11 females), median age 27 years (range: 19 to 46 years) with stable, functioning grafts (median plasma creatinine 168 μmol/liter; range: 81 to 300 μmol/liter) were studied. Median time since transplantation was eight months (range: 2 to 20 months). All patients were taking CyA orally, median dose 175 mg b.d. (range 100 to 350 mg b.d.) and prednisolone (CP Pharmaceuticals, Wrexham, UK), median dose 12.5 mg o.d. (range 8.5 to 20 mg o.d.). Eight patients were also taking nifedipine SR (Adalat Retard, Bayer UK Ltd.), median dose 30 mg b.d. (range 20 to 40 mg b.d.). Other drugs taken were ranitidine (11 patients) metoprolol (7), furosemide (5), spironolactone (4), bumetanide (3), warfarin (2), and cimetidine (1).

Patients were sampled fasting in the morning 12 hours following their previous CyA dose (the "trough" sample) and then two hours following CyA ingestion (the "peak" sample). Blood was collected, prepared and aggregation studies conducted as described in Study 2.

Eleven patients not taking nifedipine had platelet thromboxane A_2 (TXA_2) release assessed as previously described [7]. Briefly, platelet aggregation was induced as described in Study 2 by agonist concentrations (ADP 10 μmol/liter; collagen 1 mg/liter; adrenaline 5 μmol/liter), which produce maximal aggregation and therefore measurable quantities of thromboxane B_2 (TXB_2; the stable, spontaneous breakdown product of TXA_2). Three minutes after adding the agonists, the PRP was collected and added to absolute ethanol to stop the reaction. This sample was then stored at −40°C until measurement of TXB_2, in a single batch, using a specific radioimmunoassay [7].

For comparison, eight healthy subjects (Same age and sex distribution as in Study 2) were also sampled.

Study 5: Platelet function in renal allograft recipients converted from CyA to azathioprine

Renal allograft recipients (3 males, 4 females), median age 38 years (range 21 to 47 years) with stable, functioning grafts (median creatinine 133 μmol/liter; range 115 to 186 μmol/liter) were studied. Median time since transplantation was 15 months (range 12 to 17 months). Prior to changeover, all patients were taking CyA (median dose 150 mg b.d.; range 135 to 200 mg b.d.) and prednisolone (median dose 10 mg o.d.; range: 8.5 to 11 mg o.d.). Three patients were taking ranitidine and three were taking metoprolol. CyA was withdrawn over a three week period following a standard protocol. Azathioprine was introduced and the dose of prednisolone was increased temporarily during the period of conversion. Renal allograft function improved following conversion (median creatinine 106 μmol/liter; range 88 to 159 μmol/liter).

Patients were sampled while taking CyA and four to eight weeks after having ceased taking the drug, when the dose of prednisolone was again the same as pre-changeover. 'Trough' samples defined in Study 4 were collected during CyA therapy, and patients were sampled at approximately the same time in the morning when they were no longer taking this drug. Aggregation studies and platelet counts were carried out as described in Study 2.

Table 3. Median (range) percentage platelet aggregation and platelet count in PRP in samples obtained 0, 2, and 4 hours following ingestion of CyA, (15 mg/kg body weight), by normal volunteers ($N = 10$)

Sampling time	Platelet aggregation %			PRP platelet count median (*range*) × 10^9/liter
	Collagen *0.5 mg/liter*	Adrenaline *1* μmol · l^{-1}	ADP *2* μmol · l^{-1}	
0 (pre-CyA)	33 (0–80)	28 (7–78)	35 (5–87)	410 (310–480)
2 hours	46 (0–92)	52[b] (20–83)	63[a] (15–91)	350 (260–450)
4 hours	12 (0–74)	7 (0–29)	49 (0–90)	400 (490–570)

Median and (range) plasma CyA levels were 546 (212–1232) at 2 h and 560 (292–1136) ng/ml at 4 hours.

[a] $P < 0.05$ comparing pre-CyA % aggregation with % aggregation at 2 hours

[b] $P < 0.01$ comparing % aggregation at 2 hours with % aggregation at 0 and 4 hours

Other values not significant. P values were calculated using the Wilcoxon test

Whole blood platelet counts (Coulter Counter S plus) before, and six weeks after, conversion were also analyzed retrospectively in these seven patients and in an additional four patients converted from CyA. Median number of counts sampled per patient pre-conversation were 21 (range 8 to 28) and post-conversion were 9 (range 6 to 22).

Presentation of results and statistical analysis

Results are presented as median and range. In Study 1, the results in the presence of CyA were compared with those with vehicle only present, using a paired Wilcoxon rank sum test. In Study 2, the values in the baseline sample were compared with those two and four hours after the ingestion of CyA, using the same test as in Study 1. In Study 3, results were compared using a paired Wilcoxon test, since all samples were obtained, prepared and treated simultaneously. In Study 4, "peak" and "trough" samples were compared using a paired Wilcoxon test. Patient values were compared with controls using an unpaired Mann-Whitney test. In Study 5, values during CyA treatment and those after cessation of therapy were compared using a paired Wilcoxon rank sum test. All the tests used were two-tailed.

Results

Study 1: Platelet aggregation following the in vitro addition of CyA to platelets obtained from healthy volunteers

There was a significant enhancement of platelet aggregation following the addition of CyA, in vitro (Table 1). This effect was observed with all three aggregating agents, ADP, adrenaline and collagen. The enhancement was best observed at final concentrations of CyA of 250 and 500 ng/ml of PRP. CyA concentrations below 125 ng/ml did not achieve any consistent enhancement of aggregation. CyA alone did not induce any aggregation in PRP.

Table 4. Median (range) percentage aggregation (in PRP) in samples obtained at "Peak" and "Trough" CyA levels from renal allograft recipients on and off nifedipine

Aggregating agent	Patients taking CyA (N = 11)		Patients taking CyA and nifedipine (N = 8)		Controls N = 8
	Trough	Peak	Trough	Peak	
Adrenaline (μmol/ liter)					
0.2	45[a] (0–64)	64[b] (0–77)	6 (0–47)	9 (0–60)	6 (0–21)
0.5	48 (5–87)	71[a] (0–95)	44 (23–84)	56 (13–86)	20 (0–33)
5.0	65 (13–93)	←$P < 0.05$→ 78 (14–99)	78 (44–88)	73 (52–84)	66 (54–85)
Collagen (mg/liter)					
0.5	68 (0–91)	73[a] (15–93)	47 (14–80)	42 (0–77)	42 (12–60)
1.0	66 (49–97)	←$P < 0.05$→ 80 (54–95)	75 (33–93)	76 (45–86)	70 (62–84)
ADP (μmol/liter)					
1	24 (0–89)	35 (0–89)	6 (0–50)	30 (0–77)	10 (0–50)
2	61 (0–99)	74[b] (2–100)	59 (14–90)	69 (12–80)	52 (7–63)
10	64 (44–90)	←$P < 0.01$→ 83 (60–100)	69 (53–95)	68 (60–100)	75 (63–89)
PRP platelet counts median (range)$\times 10^9$/liter	260 (150–660)	280 (230–650)	330 (190–420)	290 (180–460)	360 (280–430)
Median (range) plasma CyA concn. (ng/ml)	92 (64–114)	386 (282–714)	74 (54–130)	270 (142–652)	

P values peak vs. trough concentrations as shown in table (Wilcoxon test)
[a] CyA treated vs. control: $P < 0.05$ level (Mann-Whitney test)
[b] CyA treated vs. control: $P < 0.02$ level (Mann-Whitney test)

Study 2: Platelet function following the ingestion of CyA by healthy volunteers

There was significant enhancement of platelet aggregation following the ingestion of CyA. This enhancement was no longer present 4 h after the ingestion of CyA. (Table 3)

The PRP platelet count fell at two hours and then tended to return to baseline values (Table 3). These changes, however, were not statistically significant.

The ingestion of chocolate milk drink had no effect on platelet aggregation.

Study 3: Effect of normal plasma ionic calcium concentrations and therapeutic levels of heparin on CyA-mediated enhancement of platelet aggregation, in vitro

CyA significantly enhanced platelet aggregation in heparinized PRP despite the unstable baseline tracings (**Methods**). Aggregation was considerably more enhanced in heparinized PRP when compared with citrated PRP. Thus for ADP-induced aggregation, in saline and CyA containing PRP, the percentage aggregation was significantly greater ($P < 0.01$; Wilcoxon test) in heparinized PRP. In citrated PRP, both CyA and heparin significantly enhanced platelet aggregation. However, aggregation in PRP containing both CyA and heparin was always greater than that in PRP containing the same amount of only one of these drugs. Therefore, the enhancing effects of CyA and heparin were somewhat 'additive'. PRP platelet counts were significantly lower ($P < 0.01$; Wilcoxon test) in heparinized PRP (352; 288 to 508) when compared with corresponding values in citrated PRP (462; 336 to 576). The ionic calcium concentrations were: 1.12 to 1.21 mmol/liter in heparinized PRP and 0.09 to 0.11 mmol/liter in citrated PRP.

Study 4: Platelet function in renal allograft recipients taking CyA

There was significant enhancement of platelet aggregation in the "peak" samples when compared with the "trough" samples in the patients not taking nifedipine but not in those taking this drug (Table 4).

When compared with healthy control subjects, aggregation was significantly enhanced in the patients not taking nifedipine; this enhancement was not statistically significant in the patients taking nifedipine.

PRP platelet counts were not significantly different when "trough" and "peak" counts were compared in the patient groups (Table 4). The median PRP platelet counts in the patient groups were lower than those in the healthy controls, but this did not achieve statistical significance.

When compared with control samples, platelet TXB_2 release was significantly enhanced in platelets of patients (N = 11) taking CyA only, but there was no significant difference when "peak" and "trough" samples were compared (Table 5).

Table 5. Platelet TXB_2 release in patients ($N = 11$) taking CyA only and in healthy control subjects ($N = 8$)

	ADP 10 *μmol/liter*		Adrenaline 5 *μmol/liter*		Collagen 1 *mg/liter*	
	Trough	Peak	Trough	Peak	Trough	Peak
Patients	13[b] (1.5–23)	12[b] (1–22)	18 (1.5–32.5)	18.5[a] (3–41)	11 (3.5–27)	12.5 (3–31)
Control Subjects	3 (<1–5.5)		12.5 (3–24)		17 (10–27)	

TXB_2 release is expressed as median and (range) ng TXB_2 per 10^8 platelets

[a] Control vs. CyA: $P = 0.05$, Mann-Whitney test

[b] Control vs. CyA: $P < 0.002$, Mann-Whitney test

Study 5: Platelet function in renal allograft recipients converted from CyA to azathioprine

There was a general trend for platelet aggregation to decrease following cessation of CyA therapy (Table 6). This only achieved statistical significance ($P < 0.05$) with aggregation induced by 0.5 μmol/liter adrenaline.

The PRP platelet count increased significantly ($P < 0.01$) after cessation of CyA therapy (Table 6).

Whole blood platelet counts also increased significantly ($P < 0.01$) from median 205 × 10^9/liter (range 130 to 320) before conversion to median 245 × 10^9/liter (range 170 to 380) following conversion.

Table 6. Median (range) percentage platelet aggregation (in PRP) in patients ($N = 7$) converted from CyA to azathioprine and controls ($N = 8$)

Aggregating agent	Pre-conversion	Post-conversion	Controls
Adrenaline *μmol/liter*			
0.2	13 (0–47)	0 (0–73)	6 (0–21)
0.5	52[a] ←$P < 0.05$→ (30–76)	19 (0–63)	19 (0–38)
5.0	76 (35–78)	71 (56–100)	66 (54–85)
Collagen *mg/liter*			
0.5	53 (0–62)	16 (12–85)	42 (12–60)
1.0	69 (49–85)	68 (40-79)	70 (62–84)
ADP *μmol/liter*			
1	35 (0–74)	16 (0–61)	10 (0–50)
2	64 (28–90)	54 (28–82)	52 (7–63)
10	75 (60–83)	71 (61–97)	75 (63–89)
PRP platelet counts Median (range)×10^9/liter	200 ←$P < 0.01$→ (180–260)	290 (270–500)	

Median trough pre-conversion plasma CyA was 109 (86–123) ng/ml. *P* values obtained by comparing percentage aggregation and PRP counts pre- and post-conversion (Wilcoxon test) are shown between arrows in the table. *P* values comparing controls with preconversion samples (Mann-Whitney Test) were [a] $P < 0.002$. There were no significant differences between control and post-conversion samples

Discussion

This study shows conclusively that CyA enhances platelet aggregation (and TXA_2 release) in all test systems evaluated. This supports an earlier report [3] of enhanced ADP-induced platelet aggregation in CyA-treated renal allograft recipients. These findings may have clinical implications not only with regard to the reported increased incidence of large vessel thrombosis in CyA-treated allograft recipients [3], but also in contributing to the nephrotoxic effects of the drug by promoting local vasoconstriction and glomerular arteriolar thrombosis [5, 14–17].

Platelets from healthy volunteers displayed increased activation in vitro at final concentrations of added CyA which reflected the levels achieved in clinical practice (100 to 200 ng/ml) [9]. The demonstration of CyA-mediated enhancement of aggregation in heparinized PRP shows that this phenomenon occurs at normal ionic calcium concentrations. Furthermore, the citrated-heparinized PRP experiments show that heparin- and CyA-mediated enhancement of aggregation are 'additive'. This latter observation may be of relevance since heparin alone activates platelets in patients undergoing dialysis [13]. The ingestion of a single dose of CyA in normal volunteers caused increased platelet activation, maximal at two hours but reverting to normal at four hours. This occurred in the face of a further increase in CyA concentration at four hours, and may therefore indicate that prolonged exposure to CyA is required to activate platelets consistently.

This reversal of hyperaggregability was not observed in patients taking CyA, since there was significant platelet activation at "trough" levels with an additional significant increase in aggregation at "peak" concentrations. Platelet TXA_2 release was significantly enhanced in the CyA-treated group; however, no "peak-trough" difference was observed, probably because we used agonists that induced maximal aggregation in order to achieve measurable levels of TXB_2. Nifedipine, used for the control of hypertension, prevented platelet activation at "trough" levels and blunted the "peak-trough" difference in aggregation. This effect may relate to the known inhibitory action of this drug on platelet aggregation and TXA_2 release [reviewed in 18].

Conversion of patients from CyA to azathioprine resulted in a trend towards normal platelet activity. This was accompanied by a significant rise in platelet counts, thus suggesting that CyA enhances platelet consumption.

There are many similarities between our findings and those of the Leuven group [3]. For example, they suggested a relation between CyA levels and enhanced aggregation in their patients. We showed that "peak" aggregation was greater than "trough" aggregation. They [3] did not show enhanced platelet TXA_2 release, despite reporting hyperaggregability. We also could not demonstrate enhanced TXA_2 release using collagen (the only agonist used by the Leuven group [3]), but the release of this prostanoid was markedly increased in response to ADP or adrenaline. Unimpressive enhancement of collagen-induced platelet aggregation in ex-vivo studies was a feature shared by both studies. The reason for this is not clear.

Following the initial observation [3] of an increased incidence of thrombotic episodes in the large vessels of renal allograft recipients receiving CyA, attempts to define the true incidence of this complication have been contradictory [4, 19, 20]. A number of variables identified in our study may have contributed to the inconsistency of the previous results and should

therefore be considered in future studies. For example, "peak" levels may relate to the initiation of thrombosis, and therefore the mode and frequency of administration of CyA (such as, oral, bolus i.v., and i.v. infusion) may be important in this context. Concurrent therapy with nifedipine or heparin may also modify the incidence of thrombotic complications.

The histopathology of CyA nephrotoxicity is non-specific, and debate surrounds its differentiation from allograft rejection [21, 22]. Glomerular capillary thrombosis may be a feature of rejection, but has been observed more commonly in association with CyA nephrotoxicity [5]. The mechanism of this phenomenon is not clear, but increased platelet activation, associated TXA_2-driven vasoconstriction [14] and the reduction of vascular PGI_2 (a vasodilator) and PGE_2 production reported [23–25] with CyA may play a role. In this context it is of relevance that CyA enhances TXA_2 synthesis by the kidney [26]. It is also of interest that increased urinary TXB_2 excretion has been associated with renal allograft rejection [27]. The functional importance of glomerular thrombosis in patients receiving CyA is not established, but it probably contributes to renal impairment by reducing renal blood flow producing glomerular and tubular ischemia [14, 27, 28]. The inhibition of platelet aggregation may therefore be of clinical benefit by mitigating nephrotoxicity.

The coagulation system is also altered in CyA-treated patients, and some of these changes would favor thrombosis [3]. For example, elevated plasma fibrinogen concentrations [3] increase plasma viscosity, adversely influence coagulation and enhance platelet aggregation [29].

In addition to these observations, CyA has been associated with the occurrence of a hemolytic uremic syndrome (HUS) [5, 6, 16, 17, 30, 31]. The pathogenesis of this complication is not clear but the deficiency of a prostacyclin-stimulating factor has been suggested [23, 31]. Previous observations in thrombotic thrombocytopenic purpura (TTP), a variant of HUS, have included increased platelet activation [32]. Our findings in CyA-treated patients would be consistent with a mechanism involving increased platelet activation as a contributory factor in producing this syndrome in transplant recipients.

In conclusion, CyA causes platelet activation, demonstrated in vitro, in normal volunteers and in renal allograft recipients. The effect appears to be concentration-dependent and is attenuated by concurrent nifedipine administration. This platelet activation may contribute to the nephrotoxicity of CyA treatment and to the reports of an increased incidence of thromboembolic phenomena associated with the use of this drug.

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